# Development and Validation of a Method for the Analysis of a Pharmaceutical Preparation by Near-Infrared Diffuse Reflectance Spectroscopy

MARCELO BLANCO,* JORDI COELLO, ALBA EUSTAQUIO, HORTENSIA ITURRIAGA, AND SANTIAGO MASPOCH

Contribution from *Departamento de Química, Unidad de Química Analítica, Universidad Autónoma de Barcelona, E-08193 Bellaterra, Barcelona, Spain*

Received August 17, 1998. Final revised manuscript received January 21, 1999.
Accepted for publication January 25, 1999.

**Abstract** ☐ A near-infrared (NIR) spectroscopic method based on the use of a fiber optical probe for the analysis of a commercially available pharmaceutical preparation is proposed. The analyte is identified by comparison with a second-derivative spectral library, using the correlation coefficient as the discriminating parameter. Once a sample has been positively identified, the active principle is quantified with partial least-squares (PLS) calibration. The proposed method was validated for use as a control method; to this end, the selectivity of the identification process, and the repeatability, intermediate precision, accuracy, linearity, and robustness of the active principle quantitation, were assessed.

## Introduction

"Quality" is gaining crucial significance in many fields, where it is becoming a necessity rather than a secondary goal as in former times. Achieving and ensuring quality entails strict control of raw materials, production processes (via the analysis of intermediate products), and end-products. Controlling every single step and product involved in a manufacturing process entails performing a large number of analyses. Conventional analytical methodologies involve pretreating the sample, which accounts for most of the time expended in the analysis, produces most of the errors arising during the control process, and uses substantial amounts of reagents and solvents. This has promoted the development of expeditious, reliable alternative methodologies, enabling thorough control of a production process via the simultaneous determination of several parameters.

Near-infrared (NIR) spectroscopy is gaining wide acceptance in the pharmaceutical industry as both the expeditiousness with which information can be obtained and the fact that the NIR signal depends on the chemical composition and physical properties of the sample make this technique widely applicable.[1,2] The most important physical properties that affect the spectra of pharmaceutical samples are the particle size and granulation, bringing about modifications on the spectrum like shifts or drifts of the baseline.

One of its most appealing applications of NIR is the use of a fiber optical probe coupled to the spectrophotometer to make measurements with no sample preparation, thereby avoiding the need for reagents and solvents. Although the most common use of fiber optical probes in routine analyses is the identification of raw materials,[3] its potential for quantitative analyses has also been demonstrated.[4−6]

As regards qualitative applications, NIR spectroscopy has solved various problems such as preliminary investigations in the analysis of mixtures or discrimination among similar products;[7] more widespread, however, is the identification of pure chemicals by reference to an existing spectral library.[3,4,8] In this work, we used the correlation coefficient as the discriminating parameter to identify the spectrum for the unknown sample with one in the library.

As far as quantitative analysis is concerned, NIR spectroscopy allows the determination of active principles and/or excipients in various pharmaceutical preparations by use of different multivariate calibration techniques[9] (e.g. multiple linear regression, principal component regression) of which partial least-squares (PLS) regression, used in this work, is the most widely employed choice. In quantitative analysis the modification on the spectra due to the physical properties are minimized by applying spectral pretreatments (derivatives, SNV, MSC, etc.) and/or including variability in the calibration to model it; using these procedures it is expected that the physical characteristics of the sample do not affect on the prediction capability of the model.

One more advantage of the use of NIR is the possibility to identify and quantify a sample using the same experimental data. Mathematically both methods are independent; spectral pretreatment used in each method is optimized to get the best results. For the quantification method we optimized the spectral mode to get the best prediction capability, but for the identification procedure we used the second derivative because it is the usual method that the commercial softwares includes. After defining the spectral mode needed in each method (identification or quantitative) in routine analysis, the software automatically transforms the absorbance spectral data of the recorded spectrum to the proper spectral mode previously defined for each method.

Once a new analytical protocol has been developed, it must be validated if it is to be accepted for use in routine analyses; in this way, the method is guaranteed to perform in such a way as to provide quality results every time.

There are several reported guidelines for the practical validation of analytical methods.[10,11] Official ones such as those issued by the United States Pharmacopeia (USP),[12] the International Conference on Harmonization (ICH),[13] or the Food and Drug Administration (FDA)[14] provide a framework for the validation process. As a rule, methods for regulatory submission must include studies on specificity, linearity, accuracy, precision, range, quantitation limit, and robustness.

* Corresponding author. Tel.: 34 93 581 13 67; fax: 34 93 581 24 77, e-mail: iqan8@blues.uab.es.



10.1021/js980338f CCC: $18.00
Published on Web 03/11/1999

The ICH has lately made strong harmonization efforts aimed at validating methods for the control of formulations and substances. However, chemists continue to be faced with the problem that every existing regulation of this type is concerned with the validation of chromatographic methods. The lack of officially endorsed methodologies where sample treatment differs markedly from that of a chromatographic procedure makes validation a process for which no preset rules exist.

The objective of this work is the development and validation of a NIR quantitative method. To carry out this objective, two requirements are necessary. First, the validation regulation demands a guarantee of the sample identity before quantifying the sample. Second, once a method is established, conventional tests are adapted to the specific NIR needs in order to demonstrate their suitability.

## Experimental Section

**Samples**—A pharmaceutical preparation (CBIC) commercially available in granular form contains ferrous lactate dihydrate (770 mg/g as anhydrous ferrous lactate) as active principle, and sodium croscarmellose (50 mg/g) and talc (20 mg/g) as excipients. Samples of the pure components and of the pharmaceutical preparation from different production batches were supplied by Laboratorios Menarini S.A.

**Apparatus and Software**—The spectra were recorded on a NIRSystems 5000 near-infrared spectrophotometer equipped with a reflectance detector and an AP6645 ANO3P fiber optical probe. The instrument was governed by the software package NSAS v. 3.30, from Perstorp Analytical, NIRSystems, which includes modules for acquisition and treatment of NIR spectra. It also includes IQ,[2] a program for developing the routine qualitative and quantitative analyses.

A Turbula Type T2C shaker mixer from WAB (Basel, Switzerland) was used to homogenize laboratory-made solid samples.

Calibration was performed by using the commercially available multivariate calibration software package Unscrambler v. 5.03, from Camo AS (Trondheim, Norway), which enables principal component analysis (PCA) and partial least-squares regression (PLS) and has additional capabilities for variable selection and outlier detection. The NIR spectra processed by this program were previously exported in JCAMP format from the spectrophotometer.

**Sample Preparation**—Two types of samples (laboratory and production) were used. Laboratory samples were prepared by weighing variable amounts of the active pure principle and adding different amounts of sodium croscarmellose and talc until the appropiate ferrous lactate concentration was obtained; the active principle content varied evenly over a concentration range about ±15% of the nominal value (viz. 650−850 mg/g). Each sample was shaken about 1 h and then a NIR spectrum was recorded; the shaking process was repeated again for 10 min and then a second NIR spectrum was recorded. When two consecutively recorded spectra were identical, the sample was considered homogeneous; otherwise, the shaking process was repeated.

The production samples were granulated samples obtained from different production batches, and their NIR spectra were recorded without any treatment.

**Recording of NIR Spectra**—The spectrum for each sample was recorded over the wavelength range 1100−2500 nm by inserting the fiber optical probe into the containers where the samples were received, so preparation of the sample for the analysis it was not necessary.

All samples were recorded in triplicate; after each single measurement the powder was stirred with the aid of a spatula to record different parts of the sample. The average of the three spectra for each sample was used for analysis.

Figure 1 shows the NIR spectrum for each individual component of the pharmaceutical preparation, together with that for a production sample.

**Reference Method**—The active principle in the pharmaceutical was determined by measuring the ferrous ion in ferrous lactate by redox titration[15] with $Ce^{4+}$ ion. An amount of ca. 0.2 g of sample was supplied with 100 mL of 1:50 $H_2SO_4$:$H_2O$, 0.5 g of $NaHCO_3$, and 1−2 drops of ferroin (indicator) and titrated with a previously standardized 0.1 M $Ce^{4+}$ solution.[16] The active principle content in each sample was obtained as the average of three determinations by the reference volumetric method.

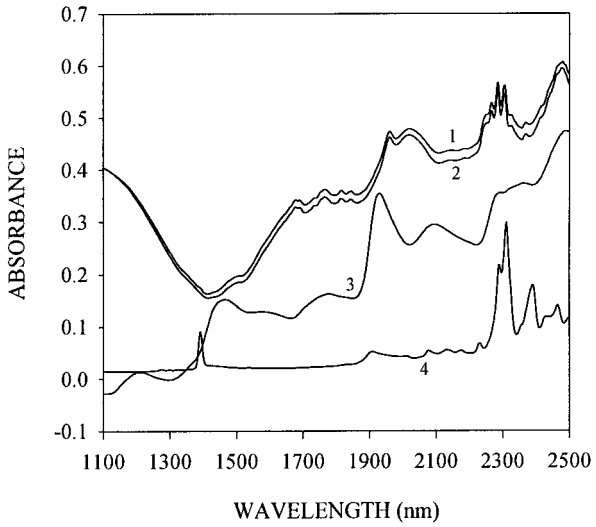


**Figure 1**—NIR spectra for a production sample and the pure components of the pharmaceutical. (1) Production sample. (2) Ferrous lactate dihydrate. (3) Sodium croscarmellose. (4) Talc.

The ferrous lactate content in the laboratory samples was calculated from weights of the pure components used to prepare them.

**Data Processing**—*Identification*—Samples were identified by comparison of their spectra with standard spectra in a reference library. To this end, a library containing spectra for the pharmaceutical preparation, the active principle, and the excipients was compiled; for each product, a set of sample spectra that met the specifications and represented all the variability in the manufacturing process were available. The library was constructed from second-derivative spectra in order to facilitate discrimination among different products and decrease spectral variability due to scattering.

Similarity between products was expressed through the correlation coefficient or match index, $\rho_{jk}$, given by

$$\rho_{jk} = \frac{\sum_{i=1}^{p}(x_{ij} - \bar{x}_j)(x_{ik} - \bar{x}_k)}{\sqrt{\sum_{i=1}^{p}(x_{ij} - \bar{x}_j)^2}\sqrt{\sum_{i=1}^{p}(x_{ik} - \bar{x}_k)^2}}$$

where the subscripts $k$ and $j$ denote the sample and product reference spectra, respectively, acquired at $p$ wavelengths, $x_{ik}$ and $x_{ij}$ are measured values of the sample and the product reference at wavelength $i$, and $\bar{x}_j$ is the value of the average spectrum $j$ over all $p$ wavelengths.

The match index ranges between −1 and +1. A value of +1 indicates perfect similarity between the unknown product and a product in the library. However, random noise associated to measurements may preclude obtainment of this value, so an identification threshold must usually be established.

*Quantitative Analysis*—All models tested were based on the PLS algorithm[17] and constructed by cross-validation, using as many segments as samples in the calibration set. All were constructed for the spectral range 1100−2200 nm and thus avoided the upper zone of the spectrum and hence the high background noise associated with the use of a fiber optical probe. The number of significant PLS components was taken to be the minimum number for which the prediction error sum of squares (PRESS) was not significantly different from the lowest PRESS value.[18]

The quality of the results was assessed in terms of the relative standard error of prediction,[19]

$$\mathrm{RSEP}(\%) = \sqrt{\frac{\sum_{i=1}^{n}(C_{\mathrm{LAB}_i} - C_{\mathrm{NIR}_i})^2}{\sum_{i=1}^{n} C_{\mathrm{LAB}_i}^2}}$$

where $C_{\mathrm{LAB}_i}$ is the reference concentration and $C_{\mathrm{NIR}_i}$ the PLS calculated concentration.

## Results and Discussion

The proposed analytical procedure uses a single spectral measurement for the simultaneous qualitative (identification) and quantitative analysis of a commercially available pharmaceutical preparation.

Unknown samples are identified by comparing their spectra with the average spectrum for each product in a library. If the sample is positively identified, then its active principle content is automatically quantified by reference to a previous PLS calibration.

After the proposed method was developed, it was validated for use as a routine control method.

**Identification**—A library consisting of 45 spectra for four different products, viz. the pharmaceutical preparation (five different CBIC production batches) and its three pure components (ferrous lactate dihydrate, sodium croscarmellose, and talc) was compiled. The spectra in the library were all recorded over the 1134−2200 nm range and converted into their second derivatives to lessen the effects of scattering. The library thus obtained exhibited no internal conflicts and correctly identified every spectrum used for self-validation.

All production samples studied (calibration and prediction sets) exhibited correlation coefficients between 0.95 and 1. An unknown sample was assumed to be positively identified if its correlation coefficient exceeded the established threshold (0.95). If any sample surpassed such a threshold for more than one product in the library, it was positively matched to that with the highest coefficient.

**Quantitative Analysis**—Available samples were split into a calibration set and a prediction set. The calibration set consisted of laboratory samples and samples from different batches of the pharmaceutical preparation (see Table 1). The laboratory samples used for calibration spanned the whole concentration range of interest in a uniform manner; these samples are powdered, and do not have the same physical properties that the real samples (granulation) have, so production samples were included in the calibration set in order to introduce the variability of the manufacturing process. Generally, a calibration set composed of only laboratory samples causes high prediction errors in production samples; in this case, using a calibration set composed of laboratory samples alone (12 samples) the prediction of production samples always gave underestimated results (except one) and a RSEP = 3.8%, an error that we considered too high.

To improve the prediction, we introduced production samples in the calibration set.[6,20] The Principal Component Analysis (PCA) was used to select the production samples to include to the calibration set. A PCA of second derivative spectra was calculated using the eight production samples available when the calibration was constructed; as can be seen from Figure 2, the samples selected were those exhibiting the greatest variability in a plot of the first component against the second, which, together, accounted for 89% of the variance. After this selection, the calibration set was composed of 17 samples, 12 of which were laboratory-made and 5 from production batches; the prediction set was composed of 19 samples, 9 laboratory-made samples, and 10 production batches.

Absorbance, first-derivative, and second-derivative PLS

**Table 1—Determination of Ferrous Lactate by Use of the Proposed NIR Spectroscopic Method and Reference Method**

| sample | reference method (mg/g) | NIR method (mg/g) |
|---|---|---|
| | Calibration Set | |
| 1 | 671 | 668 |
| 3 | 685 | 688 |
| 5 | 701 | 697 |
| 7 | 720 | 720 |
| 9 | 739 | 745 |
| 11 | 758 | 759 |
| 13 | 777 | 780 |
| 15 | 795 | 805 |
| 17 | 816 | 813 |
| 19 | 831 | 832 |
| 20 | 841 | 839 |
| 21 | 850 | 846 |
| K-4M | 791 | 784 |
| K-11 | 778 | 780 |
| K-16 | 771 | 765 |
| K-18 | 771 | 770 |
| K-19 | 754 | 757 |
| | | RSEP(C) = 0.6% |
| | Prediction Set | |
| 2 | 677 | 673 |
| 4 | 694 | 694 |
| 6 | 710 | 712 |
| 8 | 730 | 729 |
| 10 | 747 | 747 |
| 12 | 766 | 769 |
| 14 | 786 | 784 |
| 16 | 805 | 813 |
| 18 | 813 | 816 |
| K-9M | 750 | 759 |
| K-11M | 770 | 765 |
| K-12 | 777 | 765 |
| K-14 | 765 | 763 |
| K-17 | 761 | 763 |
| K-20 | 765 | 760 |
| K-21 | 756 | 760 |
| K-22 | 763 | 754 |
| K-23 | 761 | 758 |
| K-24 | 771 | 759 |
| | | RSEP(P) = 0.8% |

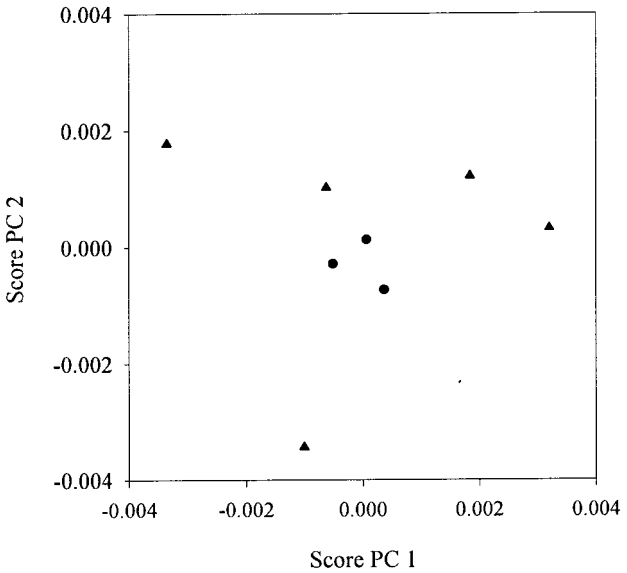


**Figure 2**—Scores plot for the first and second principal component of the production samples. (▲) Calibration samples. (●) Prediction samples.

**Table 2—Relative Standard Error of Prediction (%) for the Calibration and Prediction Sets [RSEP(C) and RSEP(P), respectively]**

| | PLS-components | RSEP(C) | RSEP(P) |
|---|---|---|---|
| absorbance | 5 | 1.2 | 2.9 |
| derivative 1 | 4 | 0.6 | 0.8 |
| derivative 2 | 5 | 0.5 | 0.9 |

**Table 3—Correlation Coefficient Found in the Identification of Unknown Samples Using the Spectral Library**

| | samples analyzed | | | |
|---|---|---|---|---|
| products included in the library | CBIC | ferrous lactate dihyd | croscarmellose sodium | talc |
| CBIC | 0.984 | 0.989 | 0.056 | 0.056 |
| ferrous lactate dihyd | 0.974 | 0.997 | –0.056 | 0.000 |
| croscarmellose sodium | 0.054 | –0.060 | 0.999 | –0.017 |
| talc | 0.049 | –0.002 | –0.012 | 0.997 |

models were tested. Table 2 shows the relative standard error of prediction (RSEP) for the different quantitative models studied. As can be seen, the best results were provided by derivative models, with no significant differences between first- and second-derivative ones. A quantitation model based on first-derivative spectra was chosen as optimal, as it was more simple; it used one fewer PLS component and gave slightly lower errors than the rest. As can be seen from the results (Table 1), this calibration model allows the accurate prediction of real-world samples with no adverse effect on the quantitation of laboratory samples.

The specification limit for acceptance of the active principle content in the pharmaceutical was ±5% of the nominal value, which is clearly larger than the prediction errors obtained with the optimum calibration procedure for the production samples; in fact, none of the samples assayed provided a relative error in excess of 1.5%. Therefore, the proposed calibration procedure is precise enough for use as a control methodology.

**Validation of the Procedure**—There is no universally accepted procedure for validating a quantitative method by NIR spectroscopy. In this work, we adapted the ICH general guidelines[13] to the purpose. The specific aspects of the NIR method that differ most markedly from the usual regulatory analysis are the use of a multivariate calibration method and the need for no sample pretreatment, i.e. sample spectra are directly recorded.

The factors considered in validating the proposed method included selectivity in the identification step, and repeatability, intermediate precision, accuracy, linearity, and robustness in the quantitation step. The concentration range spanned by the calibration is another parameter usually examined for validation; however, the use of PLS regression to determine the active principle entails the a priori selection of the concentration range of interest. During the validation process, solid evidence was obtained that the resulting quantitation errors were acceptable throughout the concentration range studied.

**Identification**—*Sensitivity*—The proposed method is able to positively identify the pharmaceutical and distinguish it from its pure components.

The identification selectivity of the proposed method was assessed by comparing the results of the NIR analysis of CBIC samples with those for the active principle and excipients in the pharmaceutical. Table 3 shows the correlations for a production sample and samples of the pure components with the different products included in the spectral library. As can be seen, the production sample was identified as CBIC (the material for which the highest correlation coefficient, in excess of 0.95, was obtained).

**Table 4—Correlation Coefficients for Different Production Batches Relative to Products in the CBIC Library and Ferrous Lactate Dihydrate**

| sample | CBIC | lactate ferrous dihydrate |
|---|---|---|
| K-17 | 0.959 | 0.955 |
| K-12 | 0.984 | 0.983 |
| K-14 | 0.977 | 0.973 |
| K-11M | 0.973 | 0.967 |
| K-20 | 0.967 | 0.964 |
| K-21 | 0.960 | 0.954 |
| K-22 | 0.959 | 0.955 |
| K-24 | 0.980 | 0.977 |
| K-9M | 0.983 | 0.972 |
| K-23 | 0.985 | 0.982 |

**Table 5—Study of Repeatability. NIR Results of 12 Analyses of the Same Production Batch**

| measurement | ferrous lactate (mg/g) |
|---|---|
| 1 | 773 |
| 2 | 775 |
| 3 | 773 |
| 4 | 771 |
| 5 | 772 |
| 6 | 777 |
| 7 | 774 |
| 8 | 778 |
| 9 | 773 |
| 10 | 777 |
| 11 | 776 |
| 12 | 775 |
| | mean: 774 |
| | CV: 0.3% |

**Table 6—Study of Intermediate Precision. NIR Results Obtained by Two Different Analysts on Three Different Days**

| sample | | day 1 | day 2 | day 3 | |
|---|---|---|---|---|---|
| 1 | operator 1 | 760 | 751 | 760 | |
| | operator 2 | 748 | 757 | 761 | $CV_{global} = 0.7\%$ |
| 2 | operator 1 | 754 | 751 | 758 | |
| | operator 2 | 749 | 763 | 755 | $CV_{global} = 0.7\%$ |

The closeness of the correlation coefficients between CBIC and ferrous lactate dihydrate reveals that the two products are very similar. To avoid conflicting identifications, Table 4 shows the correlation coefficients with CBIC and ferrous lactate dihydrate of the different production batches not used in compiling the library. No spurious identifications were made provided the established criterion was adhered to by virtue of the small spectral differences introduced by the presence of excipients in the pharmaceutical.

**Quantitative Analysis**—*Repeatability*—The repeatability of the proposed method was evaluated by performing 12 determinations of a production batch. Table 5 shows the results obtained from measurements made by the same operator on the same day. The active principle contents obtained in the 12 determinations exhibited a coefficient of variation CV = 0.3%, which is well below the usual accepted limits (<1%).

*Intermediate Precision*—The intermediate precision was assessed on two different production samples by evaluating two variable parameters in the routine control of the preparation, namely, day and operator. Table 6 shows the quantitative results obtained from measurements made by

Table 7—Study of Accuracy. NIR and Reference Results, Together with Relative Errors (%)

| sample | NIR method (mg/g) | reference method (mg/g) | relative error (%) |
|---|---|---|---|
| K-9M | 750 | 759 | –1.2 |
| K-11M | 770 | 765 | 0.6 |
| K-12 | 777 | 765 | 1.5 |
| K-14 | 765 | 763 | 0.3 |
| K-17 | 761 | 763 | –0.3 |
| K-20 | 765 | 760 | 0.6 |
| K-21 | 756 | 760 | –0.5 |
| K-22 | 763 | 754 | 1.1 |
| K-23 | 761 | 758 | 0.3 |
| K-24 | 771 | 759 | 1.5 |

two different analysts on three different days. As can be seen, the coefficient of variation for the two samples studied was slightly higher than that obtained in the repeatability study as a result of new sources of variation being introduced in the analyses; in any case, the CV values were within accepted limits for this type of test (<2%).

The variability between days and that between operators were evaluated jointly by two-way analysis of variance (ANOVA), which revealed that neither source produced any systematic errors.

*Accuracy*—Because the results could be affected by physical properties of the samples, the accuracy of the proposed procedure was only evaluated in production batches. For this purpose, the NIR results were compared with those provided by a well-characterized (reference) method.

A paired *t* test[21] was used to check whether the mean value and that held as true (viz. the average value provided by the volumetric method) were significantly different. From the results for 10 samples (Table 7), a $t_{exp}$ value of 1.41 was obtained (the $t_{tab}$ value for $P = 0.05$ and 9 degrees of freedom is 2.26). Because $t_{exp} < t_{tab}$, the results provided by NIR spectroscopy and the reference method are not significantly different.

*Linearity*—Linearity is usually estimated by evaluating the goodness of the variation of the analytical signal as a function of the analyte concentration.[10] With multivariate calibration (e.g. PLS regression), however, an alternative test suited to the methodology in question must be used. To determine the linearity of the proposed method, the NIR and reference results were compared via the following equation:

$$\text{NIR value} = a + b \times \text{reference value}$$

The samples used to check for linearity should span the whole concentration range studied, which is known to be the case with production samples only. To include samples of variable concentration with physical features as close as possible to those of the production samples, under- and overdosed samples were prepared in the laboratory. Such samples were obtained by adding variable amounts of the active principle or excipients, respectively, to samples from different production batches and homogenizing them in the shaker mixer before their NIR spectra were recorded. Their active principle contents were determined by using the reference procedure.

Table 8 shows the results for the 16 samples studied (5 of which were production samples and 11 samples used to assess linearity). As can be seen, the results provided by both techniques were quite consistent throughout the concentration range studied. A plot of NIR concentration against reference concentration was linear, with a slope of $1.03 \pm 0.06$, an intercept of $-20 \pm 51$, and a correlation coefficient $r = 0.994$. The curve has an origin and a slope

Table 8—Study of Linearity. NIR and Reference Results, Together with Relative Errors (%)

| sample | reference method (mg/g) | NIR method (mg/g) | relative error (%) |
|---|---|---|---|
| 1 | 669 | 674 | 0.7 |
| 2 | 681 | 675 | –0.9 |
| 3 | 721 | 726 | 0.7 |
| 4 | 737 | 732 | –0.7 |
| 5 | 740 | 746 | 0.8 |
| 6 | 742 | 750 | 1.0 |
| 7 | 756 | 760 | 0.5 |
| 8[a] | 761 | 758 | –0.3 |
| 9[a] | 761 | 763 | 0.3 |
| 10[a] | 765 | 763 | –0.3 |
| 11[a] | 770 | 767 | –0.4 |
| 12[a] | 770 | 765 | –0.6 |
| 13 | 794 | 799 | 0.7 |
| 14 | 804 | 818 | 1.7 |
| 15 | 832 | 835 | 0.4 |
| 16 | 837 | 841 | 0.4 |

[a] Production samples.

not significantly different from zero and one, respectivity, so the proposed method is subject to no systematic or matrix errors in relation to the reference method over the concentration range studied.[22]

Finally, a paired *t* test between the values provided by the two methods[21] was carried out in order to check whether the NIR method provided accurate results throughout the calibration range. The $t_{exp}$ value thus obtained for the samples of Table 8 was 1.45 ($t_{tab}$ for $P = 0.05$ and 15 degrees of freedom is 2.13); since $t_{exp} < t_{tab}$, the average results provided by the two methods are not significantly different.

*Robustness*—The proposed NIR method involves no sample pretreatment, so the only experimental variables potentially affecting the results are those inherent in the spectrophotometer, which are set before any spectra are recorded.

The proposed analytical method can be validated by comparing its results with those of a reference method over a period of time. The production samples analyzed in the accuracy study were manufactured over a 4-month period. Also, after the proposed method was accepted as a valid control method, an overall 10 production batches have been analyzed over a period of 8 months. The results exhibit more than acceptable accuracy (the average error relative to the theoretical value is 1.3%) and coefficients of variation (1.5%). The method is thus quite robust.

## Conclusions

A near-infrared spectroscopic method for a commercially available pharmaceutical preparation that affords direct analyses of untreated samples in the solid phase was developed. The analytical process involves the identification of the unknown sample and the quantitation of the active principle in the pharmaceutical. Identification relies on the correlation coefficient; the method allows the positive identification of the pharmaceutical and discriminates it from its pure components, and also even from the active principle, which is the major component. After the sample is identified, PLS regression allows the quantitation of the active principle with a prediction error well below the accepted limit for the pharmaceutical.

The method was validated by determining its selectivity, precision, linearity, and robustness. The results demonstrate that the proposed NIR spectroscopic method for the identification and determination of ferrous lactate in the pharmaceutical preparation CBIC is a valid alternative to

existing methods for this purpose and that it allows the successful analytical control of its production process.

## References and Notes


1. Corti, P.; Dreassi, E.; Lonardi, S. Near Infrared Reflectance Analysis: Features and Applications in Pharmaceutical and Biomedical Analysis. *Il farmaco* **1993**, *48* (1), 3−20.
2. Blanco, M.; Coello, J.; Iturriaga, H.; Maspoch, S.; de la Pezuela, C. Near-infrared spectroscopy in the pharmaceutical industry. *Analyst* **1998**, *123*, 135R−150R.
3. Khan, P. R.; Jee, R. D.; Watt, R. A.; Moffat, A. C. The identification of Active Drugs in Tablets using Near Infrared Spectroscopy. *J. Pharm. Sci.* **1997**, *3*, 447−453.
4. Blanco, M.; Coello, J.; Iturriaga, H.; Maspoch, S.; de la Pezuela, C.; Russo, E. Control Analysis of a Pharmaceutical Preparation by Near-infrared Reflectance Spectroscopy. A Comparative Study of a Spinning Module and Fibre Optic Probe. *Anal. Chim. Acta* **1994**, *298*, 183−191.
5. Blanco, M.; Coello, J.; Iturriaga, H.; Maspoch, S.; de la Pezuela, C. Strategies for Constructing the Calibration Set in the Determination of Active Principles in Pharmaceuticals by Near Infrared Diffuse Reflectance Spectrometry. *Analyst* **1997**, *112*, 761−765.
6. Blanco, M.; Coello, J.; Iturriaga, H.; Maspoch, S.; de la Pezuela, C. Quantitation of the Active Compound and Major Excipients in a Pharmaceutical Formulation by Near Infrared Diffuse Reflectance Spectroscopy with Fibre Optical Probe. *Anal. Chim. Acta* **1996**, *333*, 147−156.
7. Wu, W.; Walczak, B.; Massart, D. L.; Prebble, K. A.; Last, I. R. Spectral Transformation and Wavelength Selection in Near-infrared Spectra Classification. *Anal. Chim. Acta* **1995**, *315*, 243−255.
8. Shah, N. K.; Gemperline, P. J. Combination of the Mahalanobis Distance and Residual Variance Pattern Recognition Techniques for Classification of Near-infrared Reflectance Spectra. Anal. Chem. **1990**, *62*, 465−470.
9. Martens, H.; Naes, T. *Multivariate Calibration*; Wiley: New York, 1991.
10. Green, J. M. A Practical Guide to Analytical Method Validation. *Anal. Chem. News Features* **1996**, *68*, 305A−309A.
11. Huber, L. Validation of Analytical Methods: Review and Strategy. *LC-GC Int.* **1998**, *11* (2), 96−105.
12. *U. S. Pharmacopeia 23*; United States Pharmacopeial Convention, Inc., 1994; pp 1982−1984.
13. Harmonised Tripartite Guideline: Validation of Analytical Procedures Methodology, International Conference on Harmonisation (ICH), The European Agency for the Evaluation of Medicinal Products, London, 1996.
14. *Reviewer Guidance, Validation of Chromatographic Methods*; Center of Drug Evaluation and Research, Food and Drug Administration: Washington, D.C., 1994.
15. Vogel, A. I. *A text-book of quantitative inorganic analysis including elementary instrumental analysis*; Longman group limited: London, 1961; p 322.
16. Koltoff, I. M.; Sandell, E. B.; Meehan, E. J.; Bruckenstein, S. *Quantitative Chemical analysis*; The Macmillan Company: London, 1969; p 838.
17. Geladi, P.; Kowalski, B. Partial Least-squares Regression: a Tutorial. *Anal. Chim. Acta* **1986**, *185*, 1−17.
18. Haaland, D. M.; Thomas, E. V. Partial Least-squares Methods for Spectral Analyses. Relation to other Quantitative Calibration Methods and the Extraction of Qualitative Information. *Anal. Chem.* **1988**, *60*, 1193−1202.
19. Otto, M.; Wegscheider, W. Spectrophotometric Multicomponent Analysis Applied to Trace Metal Determinations. *Anal. Chem.* **1985**, *57*, 63−69.
20. Riley, M. R.; Arnold M. A.; Murhammer D. W. Matrix-Enhanced Calibration Procedure for Multivariate Calibration Models with Near-Infrared Spectra. *Appl. Spectrosc.* **1998**, *52* (10), 1339−1347.
21. Miller, J. C.; Miller J. N. *Statistics for analytical chemistry*; Chalmers R. A., Masson M., Eds., Ellis Horwood Series in Analytical Chemistry: Chichester, 1984; p 56.
22. Miller, J. C.; Miller J. N. *Statistics for analytical chemistry*, Chalmers R. A., Masson M., Eds., Ellis Horwood Series in Analytical Chemistry: Chichester, 1984; p 102.


## Acknowledgments


The authors are grateful to Spain's DGICyT (Project PB 96-1180) and to the Direcció General de Recerca de la Generalitat de Catalunya (1995 SGR-50) for funding this work. A. Eustaquio acknowledges additional financial support in the form of an FPI grant from the Ministerio de Educación y Ciencia. Laboratorios Menarini S.A. are also gratefully acknowledged for kindly supplying the pharmaceutical samples studied, as are our students I. Valverde and A. Villar for their cooperation in the laboratory experiments.

JS980338F